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Research Article

# Unveiling Sordariomycetes taxa associated with woody litter in Northern Thailand

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## Abstract

Sordariomycetes species are abundant in woody litter samples. In this study, we introduce two novel species, *Diaporthe thailandica* (Diaporthaceae) and *Occultitheca chiangraiensis* (Xylariaceae), from woody litter materials. We also describe a new host record of *D. tulliensis* and a new geographical record for *D. melonis*. All collections were identified based on morphology and phylogenetic analyses of combined datasets. The morphologies of the taxa fit the generic concepts of *Diaporthe* and *Occultitheca*, respectively. *Diaporthe thailandica* formed a sister clade with *D. raonikayaporum* but differs from *D. raonikayaporum* in the sizes of conidiomata, conidiogenous cells, and beta conidia. *Diaporthe thailandica* also differs from *D. raonikayaporum* by the absence of gamma conidia. *Occultitheca chiangraiensis* differs from the sister taxon *O. rosae* in having smaller ascomata and a thicker mucilaginous sheath. We also provide a synopsis of *Occultitheca* species with details on their morphology, host, and country. These findings provide valuable insights into the diversity and ecological roles of Sordariomycetes, emphasising the need for continued exploration of fungal biodiversity in various environments.

**Key words:** 2 new records, 2 new taxa, anthostomella-like taxa, phylogeny, saprobes, taxonomy

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## Introduction

Plant litter plays an important role in shaping ecological processes and supporting biodiversity, which represents a major source of organic carbon and nutrient cycling (Zhang et al. 2023). As natural decomposers, fungi hold a significant role in the breakdown of woody litter by degrading complex organic compounds efficiently (Dashtban et al. 2010; Hyde et al. 2019; Mapook et al. 2022). Although woody litter harbours various groups of fungi, comprehensive studies are limited (Hyde et al. 2020a; Phukhamsakda et al. 2022; Wanasinghe and Mortimer 2022; Xu et al. 2022; Madagammana et al. 2023).

*Diaporthe* (Diaporthaceae, Diaporthales, Sordariomycetes, Ascomycota) was established by Fuckel (1867) with *D. alnea* as the type species. *Diaporthe* is an important plant pathogen that also comprises endophytes and saprobes on a wide range of hosts (Dissanayake et al. 2017; Hyde et al. 2020b; Phukhamsakda et al. 2020; Bhunjun et al. 2022). As pathogens, *Diaporthe* cause diebacks, cankers, leaf spots, blights, melanoses, stem-end rots, and gummosis on economically and ecologically important plants (Gomes et al. 2013; Manawasinghe et al. 2021; Bhunjun et al. 2024a). The sexual morph of *Diaporthe* is characterised by immersed ascomata, erumpent pseudostroma with elongated perithecial necks, and unitunicate asci that produce hyaline ascospores (Udayanga et al. 2011; Hongsanan et al. 2023). The asexual morph has ostiolate conidiomata, cylindrical phialides, and aseptate and hyaline conidia (alpha, beta, and gamma) (Udayanga et al. 2011; Gomes et al. 2013). Molecular approaches are essential for accurate identification due to overlapping morphological characters among distinct species (Bhunjun et al. 2022; Norphanphoun et al. 2022). The taxonomy of *Diaporthe* species has been the subject of several studies (Gomes et al. 2013; Udayanga et al. 2014; Gao et al. 2017; Sun et al. 2021; Norphanphoun et al. 2022). The genus has recently been revised, and 31 species were synonymised based on multi-gene phylogeny, GCPSR (Genealogical Concordance Phylogenetic Species Recognition), as well as coalescence-based analyses of ITS, *tef1*, *tub2*, *cal*, and *his3* sequences (Dissanayake et al. 2024). Pereira and Phillips (2024) further reduced 53 species to synonymy and introduced a new species, *D. pygmaeae*, based on several molecular approaches.

*Occultitheca* (Xylariaceae, Xylariales) is characterised by immersed ascomata, short pedicellate asci with J+ apical ring, brown ascospores with hyaline dwarf cells, and a straight germ slit (Rogers and Ju 2003; Samarakoon et al. 2022). The genus is notable for having the uppermost ascospore distant from the ascus apex (Rogers and Ju 2003; Samarakoon et al. 2022). The second species, *O. rosae*, was described almost two decades later from a dead branch of *Rosa* species in Guizhou, China (Samarakoon et al. 2022). Tian et al. (2024) introduced *O. ananasi* from dead pineapple leaves from Chiang Rai, Thailand, based on morphological and molecular analyses.

Chiang Rai and Chiang Mai are located in the northern part of Thailand and are considered biodiversity hotspots (Hyde et al. 2018). During the study of woody litter microfungi in northern Thailand, two novel species and two new records were discovered based on morphology and multigene phylogeny. This underscores a critical gap in our understanding of fungal biodiversity and its ecological significance in the area, thus highlighting the need for further research (Hyde et al. 2024).

## Materials and methods

### Fungal collection, isolation, and observation

Woody litter samples were collected from forest areas in Chiang Mai and Chiang Rai Provinces, Thailand. The area is covered with a canopy of tall trees, such as dipterocarp species and *Bambusa* species. The specimens were maintained in paper bags for transport to the laboratory. The fungal

structures were observed using a Leica EZ4 stereomicroscope (Leica Microsystems (SEA) Pte Ltd, Singapore). and photographed using a Nikon ECLIPSE Ni compound microscope (Nikon, Japan) equipped with a Nikon DS-Ri2 camera. Tarosoft (R) Image Frame Work version 3.9.3.74 was used for measurements, and Adobe Photoshop CS6 software was used for the photo plates.

Single spore isolation was conducted to obtain pure culture on potato dextrose agar (PDA) as described in Senanayake et al. (2020). The culture plates were incubated at room temperature (25 ± 5 °C) for 4 weeks. Herbarium materials were deposited in the Mae Fah Luang University Fungarium (MFLU), and ex-type living cultures were deposited in the Mae Fah Luang University Culture Collection (MFLUCC) in Chiang Rai, Thailand. Index Fungorum (Index Fungorum 2024) and Facesoffungi numbers (FoF) (Jayasiri et al. 2015) were obtained. The data of the novel species will also be uploaded to the Fungalpedia website (Hyde et al. 2023).

## DNA extraction, amplification, and sequencing

Fresh mycelium was scraped from a 4-week-old culture on PDA, and DNA was extracted using the E.Z.N.A. Forensic DNA Kit (BIO-TEK) according to the manufacturer's instructions. The polymerase chain reaction (PCR) was used to amplify the ITS region (ITS5/ITS4) (White et al. 1990), *cal* (CAL228F/CAL737R) (Carbone and Kohn 1999), *his3* (CYLH3F/H3-1b) (Glass and Donaldson 1995), LSU (LR0R/LR5) (Vilgalys and Hester 1990), *rpb2* (fRPB25f/fRPB2-7cR) (Liu et al. 1999), *tef1* (EF-728F/EF-986R) (Carbone and Kohn 1999), and *tub2* (T1/T22 and BT2a/BT2b) genes (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997). The PCR conditions for each primer were set up following Samarakoon et al. (2022) and Dissanayake et al. (2024). A PCR reaction was carried out in a 25 µL reaction volume containing 12.5 µL of 2 × PCR MasterMix, 9.5 µL of double-distilled water, 1 µL of 20 µmol for each forward and reverse primer, and 1 µL of 30 ng of DNA template. The PCR products were visualised on 1% agarose gels with 6 µl of 4S green dye per 100 mL. Successful PCR products were purified and sequenced by Biogenomed Co., Ltd., South Korea. The newly generated sequences were deposited in GenBank (Tables 1, 2).

## Alignments and phylogenetic analyses

Consensus sequences were assembled using Geneious Prime 2025 (Biomatters Ltd., Auckland, New Zealand) and were used for BLASTn search against the NCBI nucleotide non-redundant database (Sayers et al. 2022). For *Diaporthe*, sequences were downloaded from GenBank (Table 1) following the classification in Dissanayake et al. (2024). For Xylariales, related sequences were downloaded from GenBank (Table 2) based on recent publications (Samarakoon et al. 2022; Sugita et al. 2022; Voglmayr et al. 2022). The sequences were aligned using MAFFT version 7 (Katoh et al. 2019) with minimal adjustment of any ambiguous nucleotides using AliView version 1.26 (Larsson 2014). The alignments were concatenated using SequenceMatrix version 1.8 (Vaidya et al. 2011).

**Table 1.** GenBank accession numbers of the taxa used in the phylogenetic analyses of *Diaporthe* section *sojae*.

| Species | Strain | ITS | *tef1* | *tub2* | *cal* | *his3* |
|---|---|---|---|---|---|---|
| *Diaporthe acaciarum* | CBS 138862$^{T}$ | KP004460 | N/A | KP004509 | N/A | KP004504 |
| *D. amaranthophila* | MAFF 246900$^{T}$ | LC459575 | LC459577 | LC459579 | LC459583 | LC459581 |
| *D. amaranthophila* | MAFF 246901 | LC459576 | LC459578 | LC459580 | LC459584 | LC459582 |
| *D. ambigua* | CBS 114015$^{T}$ | MH862953 | KC343736 | KC343978 | KC343252 | KC343494 |
| *D. ambigua* | CBS 117167 | KC343011 | KC343737 | KC343979 | KC343253 | KC343495 |
| *D. angelicae* | CBS 111592$^{T}$ | KC343027 | KC343753 | KC343995 | KC343269 | KC343511 |
| *D. angelicae* | CBS 100871 | KC343025 | KC343751 | KC343993 | KC343267 | KC343509 |
| *D. arctii* | CBS 139280$^{T}$ | KJ590736 | KJ590776 | KJ610891 | KJ612133 | KJ659218 |
| *D. arezzoensis* | MFLU 19-2880$^{T}$ | MT185503 | MT454019 | MT454055 | N/A | N/A |
| *D. batatas* | CBS 122.21$^{T}$ | KC343040 | KC343766 | KC344008 | KC343282 | KC343524 |
| *D. beilharziae* | BRIP 54792$^{T}$ | JX862529 | JX862535 | KF170921 | N/A | N/A |
| *D. biguttulata* | CFCC 52584$^{T}$ | MH121519 | MH121561 | MH121598 | MH121437 | MH121477 |
| *D. biguttulata* | CFCC 52585 | MH121520 | MH121562 | MH121599 | MH121438 | MH121478 |
| *D. brasiliensis* | CBS 133183$^{T}$ | KC343042 | KC343768 | KC344010 | KC343284 | KC343526 |
| *D. brasiliensis* | LGMF926 | KC343043 | KC343769 | KC344011 | KC343285 | KC343527 |
| *D. breyniae* | CBS 148910$^{T}$ | ON400846 | ON409188 | ON409186 | ON409189 | ON409187 |
| *D. caatingaensis* | URM7485$^{T}$ | KY085927 | KY115604 | KY115601 | KY115598 | KY115605 |
| *D. caatingaensis* | URM7484 | KY085928 | N/A | KY115602 | KY115599 | KY115606 |
| *D. caryae* | CFCC 52563$^{T}$ | MH121498 | MH121540 | MH121580 | MH121422 | MH121458 |
| *D. caryae* | CFCC 52564 | MH121499 | MH121541 | MH121581 | MH121423 | MH121459 |
| *D. chimonanthi* | SCHM 3614$^{T}$ | AY622993 | N/A | N/A | N/A | N/A |
| *D. chimonanthi* | SCHM 3603 | AY620820 | N/A | N/A | N/A | N/A |
| *D. cichorii* | MFLUCC 17-1023$^{T}$ | KY964220 | KY964176 | KY964104 | KY964133 | N/A |
| *D. cinnamomi* | CFCC 52569$^{T}$ | MH121504 | MH121546 | MH121586 | N/A | MH121464 |
| *D. cinnamomi* | CFCC 52570 | MH121505 | MH121547 | MH121587 | N/A | MH121465 |
| *D. citriasiana* | ZJUD30$^{T}$ | JQ954645 | JQ954663 | KC357459 | KC357491 | N/A |
| *D. citriasiana* | ZJUD81 | KJ490616 | KJ490495 | KJ490437 | N/A | KJ490558 |
| *D. convolvuli* | CBS 124654$^{T}$ | KC343054 | KC343780 | KC344022 | KC343296 | KC343538 |
| *D. convolvuli* | FAU649 | KJ590721 | KJ590765 | N/A | KJ612130 | KJ659210 |
| *D. coracoralinae* | URM 8912$^{T}$ | PP192078 | PP430449 | PP402241 | PP408214 | PP421133 |
| *D. coracoralinae* | FCCUFG 39 | PP192079 | N/A | PP402242 | PP408215 | PP421134 |
| *D. cucurbitae* | DAOM 42078$^{T}$ | KM453210 | KM453211 | KP118848 | N/A | KM453212 |
| *D. cucurbitae* | CBS 136.25 | KC343031 | KC343757 | KC343999 | KC343273 | KC343515 |
| *D. cuppatea* | CBS 117499$^{T}$ | KC343057 | KC343783 | KC344025 | KC343299 | KC343541 |
| *D. cyatheae* | YMJ-1364$^{T}$ | JX570889 | KC465406 | KC465403 | KC465410 | N/A |
| *D. discoidispora* | ZJUD89$^{T}$ | KJ490624 | KJ490503 | KJ490445 | N/A | KJ490566 |
| *D. discoidispora* | GZCC 22-0065 | OP056659 | OP150498 | OP150576 | OP150655 | OP150730 |
| *D. eleutharrhenae* | 01$^{T*}$ | OK017069 | OK017070 | OK017071 | N/A | N/A |
| *D. eleutharrhenae* | 02* | OK648457 | OK648458 | OK648459 | N/A | N/A |
| *D. fici-septicae* | NCYUCC 19-0108$^{T}$ | MW114349 | MW192212 | MW148269 | N/A | N/A |
| *D. fici-septicae* | MFLU 20-20178 | MW114348 | MW192211 | MW148268 | N/A | N/A |
| *D. foliorum* | CMRP 1321$^{T}$ | MT576688 | MT584310 | MT584327 | MT584341 | MT584338 |
| *D. foliorum* | CMRP 1330 | MT576671 | MT584309 | MT584328 | MT584342 | MT584340 |
| *D. ganjae* | CBS 180.91$^{T}$ | KC343112 | KC343838 | KC344080 | KC343354 | KC343596 |
| *D. ganjae* | PSCG489 | MK626955 | MK654897 | MK691287 | MK691202 | MK726204 |
| *D. goulteri* | BRIP 55657a$^{T}$ | KJ197290 | KJ197252 | KJ197270 | N/A | N/A |
| *D. gulyae* | BRIP 54025$^{T}$ | JF431299 | JN645803 | N/A | N/A | N/A |
| *D. gulyae* | BRIP 53158 | JF431284 | JN645799 | N/A | N/A | N/A |
| *D. guttulata* | CGMCC3.20100$^{T}$ | MT385950 | MT424685 | MT424705 | MW022470 | MW022491 |
| *D. guttulata* | GZCC 19-0371 | MT797178 | MT793021 | MT793032 | MW022471 | MW022492 |
| *D. helianthi* | CBS 592.81$^{T}$ | KC343115 | KC343841 | KC344083 | KC343357 | KC343599 |

| Species | Strain | ITS | *tef1* | *tub2* | *cal* | *his3* |
|---|---|---|---|---|---|---|
| *D. helianthi* | CBS 344.94 | KC343114 | KC343840 | KC344082 | KC343356 | KC343598 |
| *D. hordei* | CBS 481.92$^{T}$ | KC343120 | KC343846 | KC344088 | KC343362 | KC343604 |
| *D. infecunda* | CBS 133812$^{T}$ | KC343126 | KC343852 | KC344094 | KC343368 | KC343610 |
| *D. infertilis* | CBS 230.52$^{T}$ | KC343052 | KC343778 | KC344020 | KC343294 | KC343536 |
| *D. infertilis* | CPC 20322 | KC343053 | KC343779 | KC344021 | KC343295 | KC343537 |
| *D. juglandigena* | GUCC 422.16$^{T}$ | OP581229 | OP688534 | OP688559 | N/A | N/A |
| *D. juglandigena* | GUCC 422.161 | OP581230 | OP688535 | OP688560 | N/A | N/A |
| *D. kyushuensis* | STE-U2675$^{T}$ | AF230749 | N/A | N/A | N/A | N/A |
| *D. kyushuensis* | ch-D-1 | AB302250 | N/A | N/A | N/A | N/A |
| *D. leucospermi* | CBS 111980$^{T}$ | N/A | KY435632 | KY435673 | KY435663 | KY435653 |
| *D. leucospermi* | CAA763 | MK792291 | MK828064 | MK837915 | MK883823 | MK871433 |
| *D. longicolla* | FAU599$^{T}$ | KJ590728 | KJ590767 | KJ610883 | KJ612124 | KJ659188 |
| *D. longicolla* | CBS 100.87 | KC343196 | KC343922 | KC344164 | KC343438 | KC343680 |
| *D. longispora* | CBS 194.36$^{T}$ | KC343135 | KC343861 | KC344103 | KC343377 | KC343619 |
| *D. lusitanicae* | CBS 123213$^{T}$ | KC343137 | KC343863 | KC344105 | KC343379 | KC343621 |
| *D. lusitanicae* | CBS 123212 | KC343136 | KC343862 | KC344104 | KC343378 | KC343620 |
| *D. machilii* | SAUCC194.111$^{T}$ | MT822639 | MT855951 | MT855836 | MT855718 | MT855606 |
| *D. mayteni* | CBS 133185$^{T}$ | KC343139 | KC343865 | KC344107 | KC343381 | KC343623 |
| *D. megalospora* | CBS 143.27$^{T}$ | KC343140 | KC343866 | KC344108 | KC343382 | KC343624 |
| *D. melonis* | CBS 507.78$^{T}$ | KC343142 | KC343868 | KC344110 | KC343384 | KC343626 |
| *D. melonis* | FAU640 | KJ590702 | KJ590741 | KJ610858 | KJ612099 | KJ659184 |
| *D. melonis* | ZHKUCC 20-0014 | MT355684 | MT409338 | MT409292 | MT409314 | N/A |
| ***D. melonis*** | **MFLUCC 24-0522** | **OR936658** | **PQ774278** | **PQ774285** | **N/A** | **PQ774293** |
| ***D. melonis*** | **MFLUCC 23–0300** | **PQ777476** | **PQ774277** | **PQ774284** | **PQ774290** | **N/A** |
| *D. middletonii* | BRIP 54884e$^{T}$ | KJ197286 | KJ197248 | KJ197266 | N/A | N/A |
| *D. middletonii* | BRIP 57329 | KJ197285 | KJ197247 | KJ197265 | N/A | N/A |
| *D. minusculata* | CGMCC3.20098$^{T}$ | MT385957 | MT424692 | MT424712 | MW022475 | MW022499 |
| *D. minusculata* | GZCC 19-0345 | MT797184 | MT793027 | MT793038 | MW022476 | MW022500 |
| *D. monetii* | MF Ha18-048$^{T}$ | MW008493 | MW008515 | MW008504 | MZ671938 | MZ671964 |
| *D. monetii* | MF Ha18-049 | MW008494 | MW008516 | MW008505 | MZ671939 | MZ671965 |
| *D. morindendophytica* | ZHKUCC 22-0069$^{T}$ | ON322897 | ON315053 | ON315087 | N/A | ON315027 |
| *D. morindendophytica* | ZHKUCC 22-0070 | ON322898 | ON315054 | ON315088 | N/A | ON315028 |
| *D. myracrodruonis* | URM 7972$^{T}$ | MK205289 | MK213408 | MK205291 | MK205290 | N/A |
| *D. neoarctii* | CBS 109490$^{T}$ | KC343145 | KC343871 | KC344113 | KC343387 | KC343629 |
| *D. novem* | CBS 127270$^{T}$ | KC343156 | KC343882 | KC344124 | KC343398 | KC343640 |
| *D. novem* | CBS 127271 | KC343157 | KC343883 | KC344125 | KC343399 | KC343641 |
| *D. novem* | PL42 | JQ697843 | JQ697856 | N/A | N/A | N/A |
| *D. orixae* | KUNCC 21-10714$^{T}$ | OK283041 | N/A | N/A | OK484485 | OK484486 |
| *D. orixae* | GZCC 21-1085 | OL889852 | OL944724 | OL944726 | N/A | N/A |
| *D. ovalispora* | CGMCC3.17256$^{T}$ | KJ490628 | KJ490507 | KJ490449 | N/A | KJ490570 |
| *D. oxe* | CBS 133186$^{T}$ | KC343164 | KC343890 | KC344132 | KC343406 | KC343648 |
| *D. oxe* | CBS 133187 | KC343165 | KC343891 | KC344133 | KC343407 | KC343649 |
| *D. pachirae* | CDA 728$^{T}$ | MG559537 | MG559539 | MG559541 | MG559535 | N/A |
| *D. pachirae* | CDA 730 | MG559538 | MG559540 | MG559542 | MG559536 | N/A |
| *D. paranensis* | CBS 133184$^{T}$ | KC343171 | KC343897 | KC344139 | KC343413 | KC343655 |
| *D. paranensis* | LMICRO417 | KY461115 | KY461116 | N/A | N/A | N/A |
| *D. passiflorae* | CBS 132527$^{T}$ | JX069860 | N/A | N/A | N/A | KY435654 |
| *D. passiflorae* | CAA953 | MN190308 | MT309430 | MT309456 | MT309447 | MT309439 |
| *D. pedratalhadensis* | URM 8304$^{T}$ | PP192073 | PP430438 | PP402232 | N/A | PP421129 |
| *D. pedratalhadensis* | FCCUFG 49 | PP192075 | N/A | N/A | PP408217 | PP421131 |
| *D. phaseolorum* | AR4203$^{T}$ | KJ590738 | KJ590739 | KJ610893 | KJ612135 | KJ659220 |
| *D. pseudobauhiniae* | MFLUCC 17-1669$^{T}$ | MF190119 | MF377598 | N/A | N/A | N/A |
| *D. pseudobauhiniae* | MFLUCC 17-1670 | MF190118 | MF377599 | N/A | N/A | N/A |
| *D. quercicola* | CSUFTCC104$^{T}$ | ON076567 | ON081659 | N/A | ON081670 | ON081667 |

| Species | Strain | ITS | *tef1* | *tub2* | *cal* | *his3* |
|---|---|---|---|---|---|---|
| *D. quercicola* | CSUFTCC105 | ON076568 | ON081660 | N/A | ON081671 | ON081668 |
| *D. racemosae* | CBS 143770$^{T}$ | MG600223 | MG600225 | MG600227 | MG600219 | MG600221 |
| *D. raonikayaporum* | CBS 133182$^{T}$ | KC343188 | KC343914 | KC344156 | KC343430 | KC343672 |
| *D. raonikayaporum* | MFLUCC 14-1133 | KU712448 | KU749368 | KU743987 | KU749355 | N/A |
| *D. raonikayaporum* | MFLUCC 14-1136 | KU712449 | KU749369 | KU743988 | KU749356 | N/A |
| *D. rosae* | MFLUCC 17-2658$^{T}$ | MG828894 | N/A | MG843878 | MG829273 | N/A |
| *D. rosae* | MFLUCC 17-2574 | MG906793 | MG968954 | MG968952 | N/A | N/A |
| *D. rosiphthora* | COAD 2914$^{T}$ | N/A | QOI91674 | N/A | QOI91672 | N/A |
| *D. sackstonii* | BRIP 54669b$^{T}$ | KJ197287 | KJ197249 | KJ197267 | N/A | N/A |
| *D. schini* | CBS 133181$^{T}$ | KC343191 | KC343917 | KC344159 | KC343433 | KC343675 |
| *D. schini* | LGMF 910 | KC343192 | KC343918 | KC344160 | KC343434 | KC343676 |
| *D. schoeni* | MFLU 15-1279$^{T}$ | KY964226 | KY964182 | KY964109 | KY964139 | N/A |
| *D. schoeni* | MFLU 15-2609 | KY964229 | KY964185 | KY964112 | KY964141 | N/A |
| *D. sclerotioides* | CBS 296.67$^{T}$ | MH858974 | KC343919 | KC344161 | KC343435 | KC343677 |
| *D. sclerotioides* | CBS 710.76 | KC343194 | KC343920 | KC344162 | KC343436 | KC343678 |
| *D. serafiniae* | BRIP 55665a$^{T}$ | KJ197274 | KJ197236 | KJ197254 | N/A | N/A |
| *D. serafiniae* | BRIP 54136 | KJ197273 | KJ197235 | KJ197253 | N/A | N/A |
| *D. siamensis* | MFLUCC 10-0573a$^{T}$ | JQ619879 | JX275393 | JX275429 | JX197423 | N/A |
| *D. siamensis* | MFLUCC 12-0300 | KT459417 | KT459451 | KT459435 | KT459467 | N/A |
| *D. sojae* | FAU635$^{T}$ | KJ590719 | KJ590762 | KJ610875 | KJ612116 | KJ659208 |
| *D. sojae* | CBS 116019 | KC343175 | KC343901 | KC344143 | KC343417 | KC343659 |
| *D. stewartii* | CBS 193.36 | MH867279 | GQ250324 | JX275421 | JX197415 | N/A |
| *D. stewartii* | MN1 | KX668416 | KX852355 | N/A | N/A | N/A |
| *D. submersa* | CGMCC3.24297$^{T}$ | OP056717 | OP150556 | OP150633 | OP150710 | OP150786 |
| *D. submersa* | GZCC 22-0007 | OP056718 | OP150557 | OP150634 | OP150711 | OP150787 |
| *D. subordinaria* | CBS 464.90$^{T}$ | KC343214 | KC343940 | KC344182 | KC343456 | KC343698 |
| *D. subordinaria* | CBS 101711 | KC343213 | KC343939 | KC344181 | KC343455 | KC343697 |
| *D. tarchonanthi* | CBS 146073$^{T}$ | MT223794 | N/A | MT223733 | N/A | MT223759 |
| *D. tecomae* | CBS 100547$^{T}$ | KC343215 | KC343941 | KC344183 | KC343457 | KC343699 |
| *D. tectoendophytica* | MFLUCC 13-0471$^{T}$ | KU712439 | KU749367 | KU743986 | KU749354 | N/A |
| *D. tectonendophytica* | LC8115 | KY491550 | KY491560 | KY491570 | N/A | N/A |
| *D. terebinthifolii* | CBS 133180$^{T}$ | KC343216 | KC343942 | KC344184 | KC343458 | KC343700 |
| *D. terebinthifolii* | LGMF907 | KC343217 | KC343943 | KC344185 | KC343459 | KC343701 |
| ***D. thailandica*** | **MFLUCC 24-0523$^{T}$** | **OR946374** | **PQ774276** | **PQ774283** | **N/A** | **PQ774292** |
| ***D. thailandica*** | **MFLUCC 23–0299** | **PQ777475** | **PQ774275** | **PQ774282** | **PQ774289** | **N/A** |
| *D. thunbergiicola* | MFLUCC 12-0033$^{T}$ | KP715097 | KP715098 | N/A | N/A | N/A |
| *D. tulliensis* | BRIP 62248a$^{T}$ | KR936130 | KR936133 | KR936132 | N/A | N/A |
| *D. tulliensis* | JZB320128 | MK335814 | MK523573 | MK500152 | MK500240 | N/A |
| *D. tulliensis* | MFLUCC 14-1139 | KU712438 | KU749366 | KU743985 | KU749353 | N/A |
| ***D. tulliensis*** | **MFLUCC 24-0524** | **PQ777478** | **PQ774280** | **PQ774287** | **N/A** | **PQ774294** |
| ***D. tulliensis*** | **MFLUCC 23–0301** | **PQ777477** | **PQ774279** | **PQ774286** | **PQ774291** | **N/A** |
| *D. ueckerae* | FAU656$^{T}$ | KJ590726 | KJ590747 | KJ610881 | KJ612122 | KJ659215 |
| *D. ueckerae* | BRIP 54736j | KJ197282 | KJ197244 | KJ197262 | N/A | N/A |
| *D. unshiuensis* | ZJUD50$^{T}$ | KJ490585 | KJ490464 | KJ490406 | N/A | KJ490527 |
| *D. unshiuensis* | PSCG339 | MK626928 | MK654879 | MK691300 | MK691181 | MK726188 |
| *D. vangoghii* | MF Ha18-045$^{T}$ | MW008491 | MW008513 | MW008502 | MZ671936 | MZ671962 |
| *D. vangoghii* | MF Ha18-046 | MW008492 | MW008514 | MW008503 | MZ671937 | MZ671963 |
| *D. vargemgrandensis* | URM 8784$^{T}$ | PP192069 | PP430456 | PP421092 | PP421068 | PP421135 |
| *D. vexans* | CBS 127.14 | KC343229 | KC343955 | KC344197 | KC343471 | KC343713 |
| *D. vexans* | FAU597 | KJ590734 | KJ590774 | KJ610889 | KJ612131 | KJ659216 |
| *D. vochysiae* | LGMF1583$^{T}$ | MG976391 | MK007526 | MK007527 | MK007528 | MK033323 |
| *D. yunnanensis* | CGMCC3.18289$^{T}$ | KX986796 | KX999188 | KX999228 | KX999290 | KX999267 |
| *D. yunnanensis* | LC8107 | KY491542 | KY491552 | KY491562 | KY491572 | N/A |

The newly generated sequences are in bold. Type and reference collections are denoted with "T," while missing data are shown as "N/A". * The species name is invalid but included for taxon sampling.

**Table 2.** GenBank accession numbers of the taxa used in the phylogenetic analyses of Xylariales.

| Species | Strain | ITS | LSU | *rpb2* | *tub2* |
|---|---|---|---|---|---|
| *Albicollum vincensii* | CBS 147286$^{T}$ | ON869297 | ON869297 | ON808475 | ON808519 |
| *Amphirosellinia nigrospora* | HAST 91092308$^{T}$ | GU322457 | N/A | GQ848340 | GQ495951 |
| *Anthostomella helicofissa* | MFLUCC 14-0173$^{T}$ | MW240653 | MW240583 | KP340534 | KP406617 |
| *Anthostomella lamiacearum* | MFLU18-0101$^{T}$ | MW240669 | MW240599 | MW658648 | N/A |
| *Anthostomelloides brabeji* | CBS 110128 | EU552098 | EU552098 | N/A | N/A |
| *Anthostomelloides krabiensis* | MFLUCC 15-0678$^{T}$ | KX305927 | KX305928 | KX305929 | N/A |
| *Anthostomelloides leucospermi* | CBS 110126$^{T}$ | EU552100 | EU552100 | N/A | N/A |
| *Barrmaelia macrospora* | CBS 142768$^{T}$ | KC774566 | KC774566 | MF488995 | MF489014 |
| *Biscogniauxia nummularia* | MUCL 51395$^{T}$ | KY610382 | KY610427 | KY624236 | KX271241 |
| *Chaetomium elatum* | CBS 374.66 | KC109758 | KC109758 | KF001820 | KC109776 |
| *Circinotrichum circinatum* | CBS 148326 | ON400743 | ON400796 | ON399328 | N/A |
| *Circinotrichum maculiforme* | CBS 140016$^{T}$ | KR611874 | KR611895 | ON399338 | N/A |
| *Clypeosphaeria mamillana* | CBS 140735$^{T}$ | KT949897 | KT949897 | MF489001 | MH704637 |
| *Clypeosphaeria mamillana* | WU 33599 | KT949898 | KT949898 | N/A | N/A |
| *Clypeosphaeria oleae* | CPC 36779 | MN562130 | MN567637 | N/A | N/A |
| *Coniocessia maxima* | CBS 593.74$^{T}$ | GU553332 | MH878275 | N/A | N/A |
| *Coniocessia nodulisporioides* | CBS 281.77$^{T}$ | MH861061 | MH872831 | N/A | N/A |
| *Dematophora bunodes* | CBS 124028 | MN984619 | MN984625 | N/A | MN987245 |
| *Didymobotryum rigidum* | JCM 8837$^{T}$ | LC228650 | LC228707 | N/A | N/A |
| *Digitodochium amoenum* | CBS 147285$^{T}$ | ON869303 | ON869303 | ON808481 | ON808525 |
| *Digitodochium rhodoleucum* | NBRC 32296 | LC146732 | LC146732 | N/A | N/A |
| *Emarcea castanopsidicola* | CBS 117105 | AY603496 | MK762717 | MK791285 | MK776962 |
| *Emarcea eucalyptigena* | CBS 139908 | KR476733 | MK762718 | MK791286 | MK776963 |
| *Entalbostroma erumpens* | ICMP 21152$^{T}$ | KX258206 | N/A | KX258204 | KX258205 |
| *Entoleuca mammata* | JDR 100 | GU300072 | N/A | GQ844782 | GQ470230 |
| *Entosordaria perfidiosa* | CBS 142773$^{T}$ | MF488993 | MF488993 | MF489003 | MF489021 |
| *Fasciatispora arengae* | MFLUCC 15-0326a$^{T}$ | MK120275 | MK120300 | MK890794 | MK890793 |
| *Fasciatispora cocoes* | MFLUCC 18-1445$^{T}$ | MN482680 | MN482675 | MN481517 | MN505154 |
| *Graphostroma platystomum* | CBS 270.87$^{T}$ | JX658535 | DQ836906 | KY624296 | HG934108 |
| *Gyrothrix verticillata* | CBS 148806 | ON400759 | ON400813 | ON399318 | N/A |
| *Halorosellinia krabiensis* | MFLU 17-2596$^{T}$ | MN047119 | MN017883 | N/A | MN431493 |
| *Hansfordia pruni* | CBS 194.56$^{T}$ | MK442585 | MH869122 | KU684307 | N/A |
| *Hansfordia pulvinata* | CBS 144422 | MK442587 | MK442527 | N/A | N/A |
| *Helicogermslita clypeata* | MFLU 18-0852$^{T}$ | MW240666 | MW240596 | MW658647 | MW775614 |
| *Hypocopra rostrata* | NRRL 66178 | KM067909 | KM067909 | N/A | N/A |
| *Hypocopra zeae* | MFLU 18-0809$^{T}$ | MW240671 | MW240601 | MW658650 | MW775616 |
| *Hypocreodendron sanguineum* | J.D.R.169$^{T}$ | GU322433 | N/A | GQ844819 | GQ487710 |
| *Hypomontagnella monticulosa* | MFLUCC 18-0362 | MN337231 | MN336235.2 | MN366246 | MN509783 |
| *Muscodor thailandicus* | MFLUCC 17-2669 | MK762707 | MK762714 | MK791283 | MK776960 |
| *Muscodor ziziphi* | MFLUCC 17-2662 | MK762705 | MK762712 | MK791281 | MK776958 |
| *Jackrogersella multiformis* | CBS 119016$^{T}$ | KC477234 | KY610473 | KY624290 | KX271262 |
| *Kretzschmaria deusta* | CBS 163.93 | KC477237 | KY610458 | KY624227 | KX271251 |
| *Kretzschmariella culmorum* | JDR 88 | KX430043 | N/A | KX430045 | KX430046 |
| *Linosporopsis ischnotheca* | CBS 145761$^{T}$ | MN818952 | MN818952 | MN820708 | MN820715 |
| *Magnostiolata mucida* | MFLU 19-2133$^{T}$ | MW240673 | MW240603 | MW658652 | MW775618 |
| *Melanographium phoenicis* | MFLUCC 18-1481$^{T}$ | MN482677 | MN482678 | N/A | N/A |
| *Melanographium smilacis* | MFLU 21-0075$^{T}$ | MZ538514 | MZ538548 | N/A | N/A |
| *Nemania serpens* | HAST 235 | GU292820 | N/A | GQ844773 | GQ470223 |
| *Neoanthostomella fici* | MFLU 19-2765$^{T}$ | MW114390 | MW114445 | MW177711 | N/A |
| *Neoxylaria juruensis* | HAST 92042501 | GU322439 | N/A | GQ844825 | GQ495932 |
| *Nigropunctata bambusicola* | MFLU 19-2145$^{T}$ | MW240664 | MW240594 | MW658646 | N/A |
| *Nigropunctata nigrocircularis* | MFLU 19-2130$^{T}$ | MW240661 | MW240591 | N/A | MW775612 |
| *Occultitheca ananasi* | MFLU 23-0251$^{T}$ | OR438426 | OR438886 | N/A | N/A |
| *Occultitheca ananasi* | MFLUCC 23-0120 | OR438427 | OR438887 | OR634962 | OR538094 |

| Species | Strain | ITS | LSU | *rpb2* | *tub2* |
|---|---|---|---|---|---|
| ***Occultitheca chiangraiensis*** | **MFLU 24-0414$^{T}$** | **PQ777479** | **PQ778042** | **PQ774295** | **PQ774288** |
| *Occultitheca rosae* | HKAS 102393$^{T}$ | MW240672 | MW240602 | MW658651 | MW775617 |
| *Podosordaria mexicana* | WSP 176 | GU324762 | N/A | GQ853039 | GQ844840 |
| *Poronia punctata* | CBS 656.78$^{T}$ | KT281904 | KY610496 | KY624278 | KX271281 |
| *Pseudoanthostomella pini-nigrae* | MFLUCC 16-0478$^{T}$ | KX533453 | KX533454 | KX789492 | N/A |
| *Pseudoceratocladium polysetosum* | FMR 10750$^{T}$ | KY853430 | KY853490 | ON399348 | N/A |
| *Rosellinia chiangmaiensis* | MFLUCC 15-0015$^{T}$ | KU246226 | KU246227 | N/A | N/A |
| *Rosellinia lamprostoma* | HAST 89112602 | EF026118 | N/A | GQ844778 | EF025604 |
| *Sarcoxylon compunctum* | CBS 359.61 | KT281903 | KT281898 | KY624230 | KX271255 |
| *Sordaria fimicola* | CBS 723.96 | MH862606 | MH874231 | DQ368647 | N/A |
| *Spiririma gaudefroyi* | CBS 147284$^{T}$ | ON869320 | ON869320 | ON808497 | ON808541 |
| *Spirodecospora melnikii* | MAFF 247746$^{T}$ | LC731937 | LC731946 | LC731955 | N/A |
| *Spirodecospora paulospiralis* | MAFF 247749$^{T}$ | LC731940 | LC731949 | LC731957 | N/A |
| *Stromatoneurospora phoenix* | BCC 82040 | MT703666 | MT735133 | MT742605 | MT700438 |
| *Vamsapriya indica* | MFLUCC 12-0544 | KM462839 | KM462840 | KM462841 | KM462838 |
| *Xenoanthostomella chromolaenae* | MFLUCC 17-1484$^{T}$ | MN638863 | MN638848 | MN648729 | N/A |
| *Xenoanthostomella cycadis* | CBS 137969$^{T}$ | KJ869121 | KJ869178 | ON399350 | N/A |
| *Xylaria acuminatilongissima* | HAST 95060506$^{T}$ | EU178738 | N/A | GQ853028 | GQ502711 |
| *Xylaria arbuscula* | CBS 126415 | KY610394 | KY610463 | KY624287 | KX271257 |
| *Xylaria botuliformis* | HAST 89091627 | MN089652 | N/A | MN095399 | MN095400 |
| *Xylaria brunneovinosa* | HAST 720$^{T}$ | EU179862 | N/A | GQ853023 | GQ502706 |
| *Xylaria ellisii* | DAOM 628556$^{T}$ | MN218820 | MN218817 | MN216186 | N/A |
| *Xylaria eucalypti* | CPC 36723 | MN562127 | MN567634 | N/A | N/A |
| *Xylaria hypoxylon* | CBS 122620$^{T}$ | AM993141 | KM186301 | KM186302 | KM186300 |

The newly generated sequences are in bold. Type and reference collections are denoted with "T," while missing data are shown as "N/A".

Maximum likelihood analyses (ML), including 1000 bootstrap pseudoreplicates, were performed at the CIPRES web portal (Miller et al. 2017) using RAxML version 8.2.12 (Stamatakis 2014). The general time reversible (GTR) model with a discrete gamma distribution plus invariant site (GTR + I + G) was used as the nucleotide substitution model. The best model for each gene was determined in JModelTest version 2.1.10 (Darriba et al. 2012) for the Bayesian analysis. The Bayesian inference posterior probabilities (BPP) distribution (Zhaxybayeva and Gogarten 2002) was estimated by Markov Chain Monte Carlo sampling (MCMC) in MrBayes version 3.2.2 on XSEDE (Ronquist et al. 2012) with four runs of MCMC for 1,000,000 generations, sampling trees every 100$^{th}$ generation. The first 25% of trees were excluded as burn-in, and the remaining trees were used to calculate posterior probabilities (BPP). The trees were visualised using FigTree version 1.4.4 (Rambaut 2012) and edited using Adobe Illustrator® CS6 (Adobe Systems, USA).

## Genealogical concordance phylogenetic species recognition analysis

The closely related strains were further analysed using the genetic distances by performing a pairwise homoplasy index test (Φw) (Taylor et al. 2000; Bruen et al. 2006). A pairwise homoplasy index (PHI) test was performed in SplitsTree (CE 6.0.0) using Kimura's two-parameter (K2P) models for low genetic distance datasets (Huson and Bryant 2024). LogDet transformation was applied for the average of nucleotide frequencies and splits decomposition graph options (Gu and Li 1996a, b; Taylor et al. 2000; Bruen et al. 2006; Huson and Bryant 2006; Gioan and Paul 2012; Nishimaki and Sato 2019). The standard deviation of split frequencies PHI test result (Φw) < 0.05 indicates significant recombination within the dataset.

## Results

### Phylogenetic analyses of section *sojae*

The phylogeny represents taxa from section *sojae* based on the concatenated dataset of ITS, *tef1*, *tub2*, *cal*, and *his3* sequences. The combined sequence alignment comprised 159 taxa with 3279 characters, including gaps (ITS: 1–587, *tef1*: 588–1131, *tub2*: 1132–2067, *cal*: 2068–2682, *his3*: 2683–3279). The ML and BI analyses showed similar topologies (Fig. 1). The best scoring ML tree had a final likelihood value of -47785.673. The matrix had 1463 constant sites, 1408 parsimony informative sites, and 2202 distinct site patterns. Estimated base frequencies were as follows: A = 0.213, C = 0.325, G = 0.239, T = 0.224, substitution rates: AC = 1.16629, AG = 3.42597, AT = 1.16629, CG = 1.00000, CT = 4.44139, GT = 1.000, gamma distribution shape parameter = 0.954, and tree length = 7.506.

**Sordariomycetes O.E. Erikss. & Winka**
**Diaporthales Nannf.**
**Diaporthaceae Höhn. ex Wehm.**

***Diaporthe melonis* Beraha & M.J. O'Brien, Phytopath. Z. 94(3): 205 (1979)**
Index Fungorum: IF312933
Facesoffungi Number: FoF17285
Fig. 2

**Description.** ***Saprobic*** on dead unidentified branch. ***Sexual morph*:** not observed. ***Asexual morph*: *Conidiomata*** 148–374 × 128–338 µm high ($\bar{x}$ = 250 × 225 µm, n = 15), pycnidial, mostly scattered, immersed, slightly erumpent through the host surface, discoid or subglobose, with a solitary undivided locule. ***Conidiophores*** reduced to conidiogenous cells. ***Alpha conidiogenous cells*** 5.7–25 × 1.1–2.5 µm ($\bar{x}$ = 15.6 × 1.7 µm, n = 50), hyaline, rarely branched, mostly aseptate, densely aggregated, cylindrical, straight to slightly curved and smooth. ***Alpha conidia*** 5–7.3 × 1.9–2.7 µm ($\bar{x}$ = 6.3 × 2.3 µm, n = 40), unicellular, fusiform to ellipsoidal, apex and base rounded, hyaline, smooth, bi-guttulate. ***Beta conidiogenous cells*** 6.2–16 × 1.6–2.6 µm ($\bar{x}$ = 9.4 × 2.1 µm, n = 40), phialidic, subcylindrical, tapering towards the apex, hyaline. ***Beta conidia*** 19–27 × 1–2 µm ($\bar{x}$ = 23 × 1.5 µm, n = 40), filiform, aseptate, hyaline, smooth-walled, straight from base, and curve at apex. ***Gamma conidia*** not observed.

**Culture characteristics.** Colonies on PDA, reaching 20 mm diam., after 3 weeks at 25 °C, initially white, turning beige after 7–10 days, flat, felty with a thick texture at the centre and marginal area, lacking aerial mycelium; reverse, glossy grey, radiating outwardly.

**Material examined.** Thailand, Chiang Rai Province, Muang District, on a dead unidentified dicot branch, 16 January 2023, J. Louangphan, CR1-02 (MFLU 23–0474); living culture MFLUCC 24–0522 = MFLUCC 23–0300.

**Hosts.** *Annona squamosa* (Annonaceae), *Berberis aristata* (Berberidaceae), *Carapa guianensis* (Meliaceae), *Citrus grandis* cv. *Tomentosa* (Rutaceae), *Cucumis melo* (Cucurbitaceae), *Glottidium* sp. (Fabaceae), *Glycine max*, *G. soja* (Fabaceae), unidentified branch (Dong et al. 2021a, b; Hongsanan et al. 2023; This study).

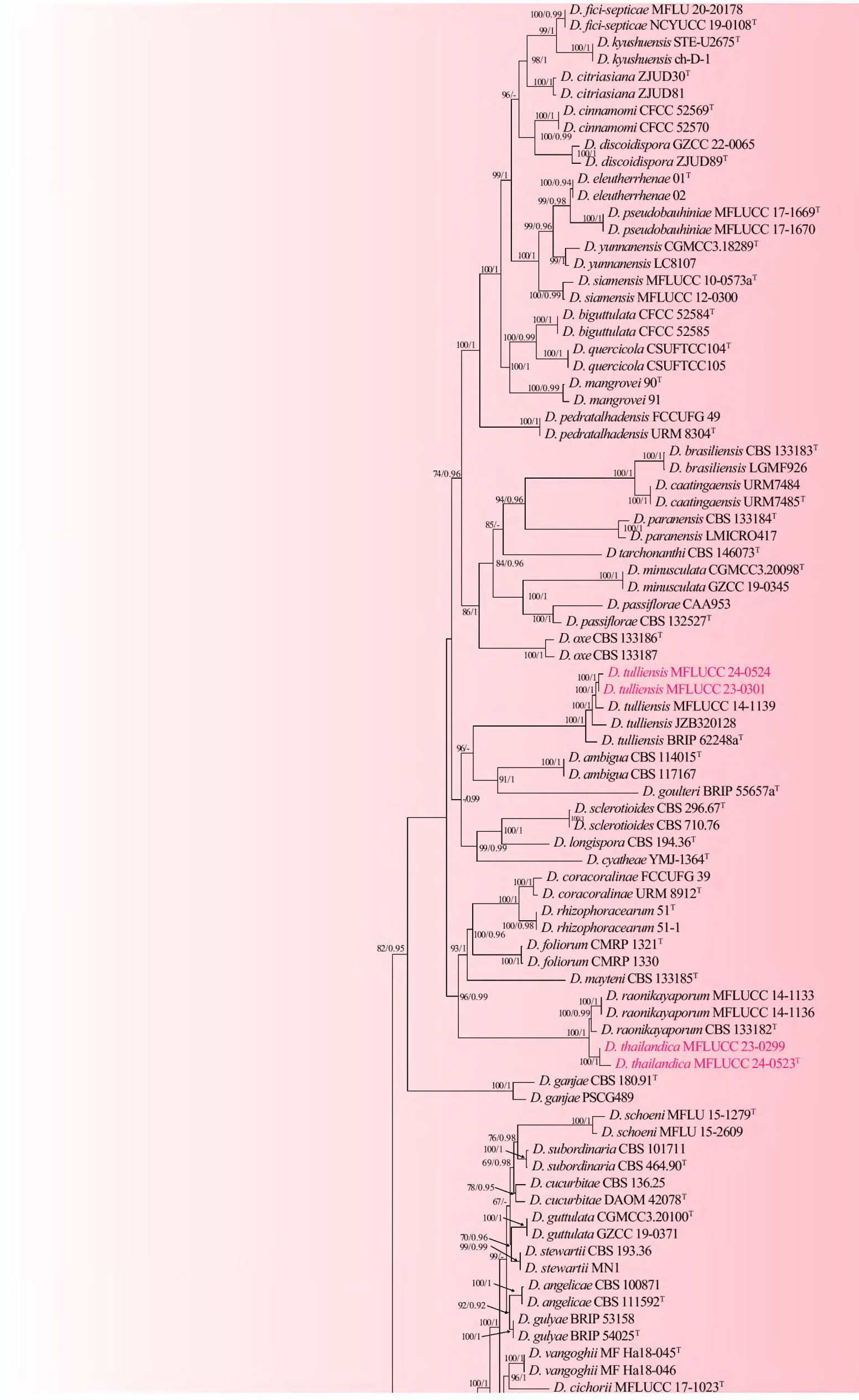


**Figure 1.** Phylogram of the *Diaporthe sojae* species complex generated from a maximum likelihood analysis based on the combined ITS, *tef1*, *tub2*, *cal,* and *his3* sequence data. *Diaporthella corylina* (CBS 121124) was used as the outgroup. Bootstrap support values ≥ 60% ML/≥0.90 BPP are given at the nodes. The newly generated taxa are indicated in red. The holotype/ex-type strains are denoted with T.

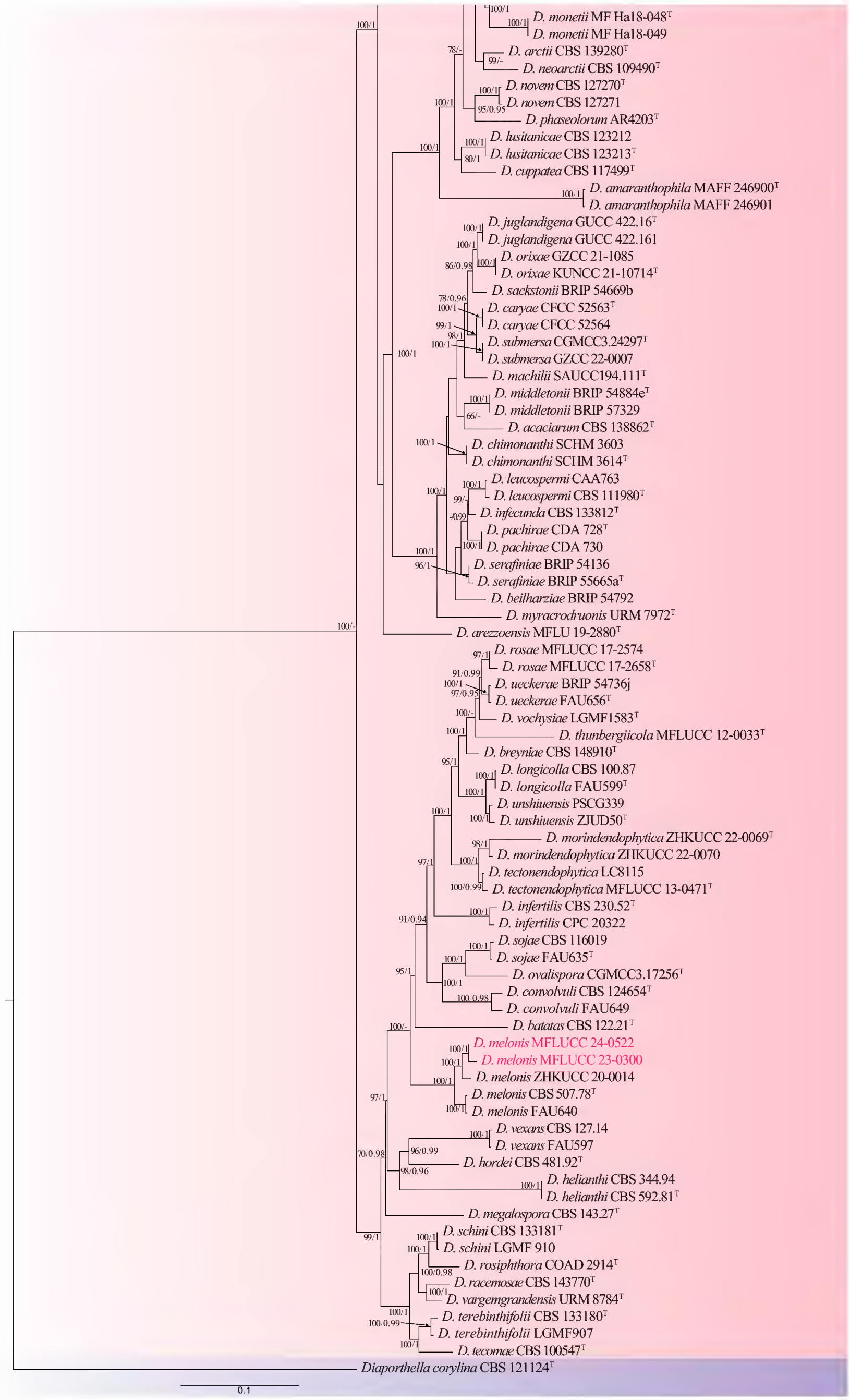


**Figure 1.** Continued.

**Figure 2.** *Diaporthe melonis* (MFLU 23–0474) **a** host substrate **b** conidiomata on substrate **c** transverse section of conidioma **d, e** vertical section through conidiomata **f, g** conidiophores and conidiogenous cells **h, i** beta conidia **j** alpha conidia **k** a germinated conidium **l** front and reverse view of the colony on PDA. Scale bars: 200 µm (**b–d**); 100 µm (**e**); 20 µm (**f, k**); 10 µm (**g, h–j**).

**Distribution.** China, Myanmar, India, Indonesia, Japan, Thailand, the United States (Dong et al. 2021a, b; Hongsanan et al. 2023; this study).

**Notes.** Our isolates (MFLUCC 23–0300 and MFLUCC 24–0522) clustered with *D. melonis* isolates (CBS 507.78, FAU640, and ZHKUCC 20-0014) with 100% ML/1.00 BPP support (Fig. 1). Our isolate has a similar morphology to *D. melonis* but differs in having smaller conidiomata (148–374 µm vs. 100–500 µm diam.) and smaller alpha conidia (6.3 × 2.3 µm vs. 8.3 × 2.6 µm) (Beraha and O'Brien 1979). Our isolate has a beige culture compared to the brown culture of *D. melonis* (Beraha and O'Brien 1979). Our isolate also differs from *D. melonis* (*D. guangdongensis* ZHKUCC 20-0014) in the size of conidiomata (128–338 × 148–374 µm *vs.* 130–515 × 100–390 µm), alpha conidia (5–7.3 × 1.9–2.7 µm vs. 6–8 × 2–4 µm), and beta conidia (19–27 × 1–2 µm vs. 14–35 × 1–2 µm) (Dong et al. 2021a, b). Therefore, we report our isolate as a new geographical record of *D. melonis* from Thailand.

***Diaporthe thailandica*** **Louangphan, Phukhams., K.D. Hyde & Bhunjun, sp. nov.**

Index Fungorum: IF903043

Facesoffungi Number: FoF17286

Figs 3, 4

**Etymology.** The name refers to the country where the holotype was collected.

**Holotype.** MFLU 23–0473.

**Description.** ***Saprobic*** on decaying dicot, visible as black necks immerging through the host surface. ***Sexual morph*****: *Ascomata*** 328–495 × 303–371 µm ($\bar{x}$ = 400 × 343 µm, n = 10), immersed in the host epidermis, globose to sub-globose, solitary or occur in clusters, black, ostiolate, papillate. ***Ostiole neck*** 220 × 86 µm, long, filled with periphysate. ***Peridium*** 20–50 µm wide, composed of several layers of cells of *textura angularis*, outer layers dark brown and inner layers hyaline to brown, thin-walled. ***Paraphyses*** 3.2–6.6 µm (n = 20), thin-walled, 2–4-septate, hyaline, wide at base, tapering towards the apex. ***Asci*** 45–58.9 × 8.6–12.7 µm ($\bar{x}$ = 51.5 × 10.5 µm, n = 40), unitunicate, 8-spored, clavate to subclavate, straight to slightly curved, sessile, with a J-, apical ring. ***Ascospores*** 11–15.5 × 3.9–5.6 µm ($\bar{x}$ = 13.5 × 4.7 µm, n = 40) L/W = 2.8, overlapping uniseriate to biseriate, 1-septate, constricted at the septum, ellipsoidal, smooth-walled, 2–4-guttulate, straight, hyaline, without appendages or a mucilaginous sheath. ***Asexual morph*** on PDA: ***Conidiomata*** 500–700 × 300–600 µm ($\bar{x}$ = 580 × 480 µm, n = 10), pycnidial, scattered or aggregated, globose or variable in shape, ostiolate with prominent neck, dark brown to black, pycnidal wall brown, consisting of thick-walled cells of *textura angularis*, conidial mass globose, initially hyaline to yellowish, becoming white to cream, conidial droplets exuding from central ostioles. ***Conidiophores*** 10–29.5 × 1.3–2.5 µm ($\bar{x}$ = 16.9 × 1.9 µm, n = 40), ampulliform to subcylindrical, filiform, branched to unbranched, 1–3-septate, hyaline, smooth, straight or slightly curved, wider at base, tapering towards the apex. ***Conidiogenous cells*** 2.1–8.1 × 1–2.3 µm ($\bar{x}$ = 4.2 × 1.5 µm, n = 40), subcylindrical, filiform, straight to curved, tapering towards the apex, collarette not flared, hyaline. ***Alpha conidia*** 5.3–8.8 × 2.3–3.5 µm ($\bar{x}$ = 7.3 × 2.9 µm, n = 40), ellipsoid, apex bluntly rounded, base obtuse to subtruncate, smooth, hyaline, bi- to multi-guttulate. ***Beta conidia*** 8.5–18.5 × 1.2–2 µm ($\bar{x}$ = 13.3 × 1.7 µm, n = 40), filiform, flexible to slightly curved, hyaline, base subtruncate, and aseptate. ***Gamma conidia*** not observed.

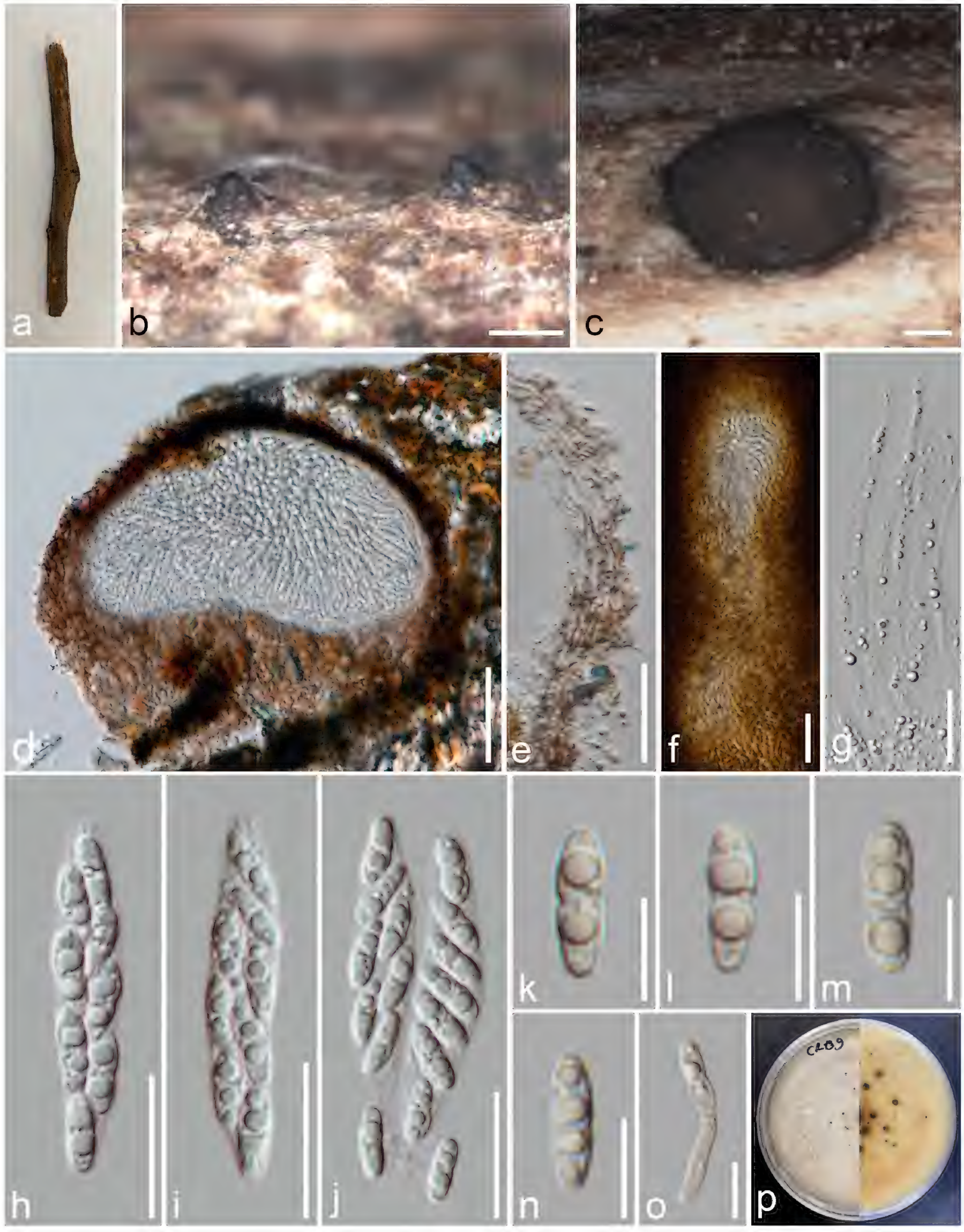


**Figure 3.** *Diaporthe thailandica* (MFLU 23–0473, holotype) **a** host substrate **b, c** ascomata on host substrate **d** vertical section through ascoma **e** peridium **f** ostiole **g** hamathecium **h–j** asci **k–n** ascospores **o** a germinated ascospore **p** front and reverse view of the colony on PDA. Scale bars: 200 µm (**b**); 100 µm (**c, d**); 20 µm (**e–j**); 10 µm (**k–o**).

**Culture characteristics.** Colonies on PDA, reaching 40 mm diam., after 2 weeks at 25 °C, initially white, turning pale brown after 7–10 days, radiating to the edge, margin undulate, medium dense, flat or umbonate; reverse, cream, radiating white outwardly with grey patches.

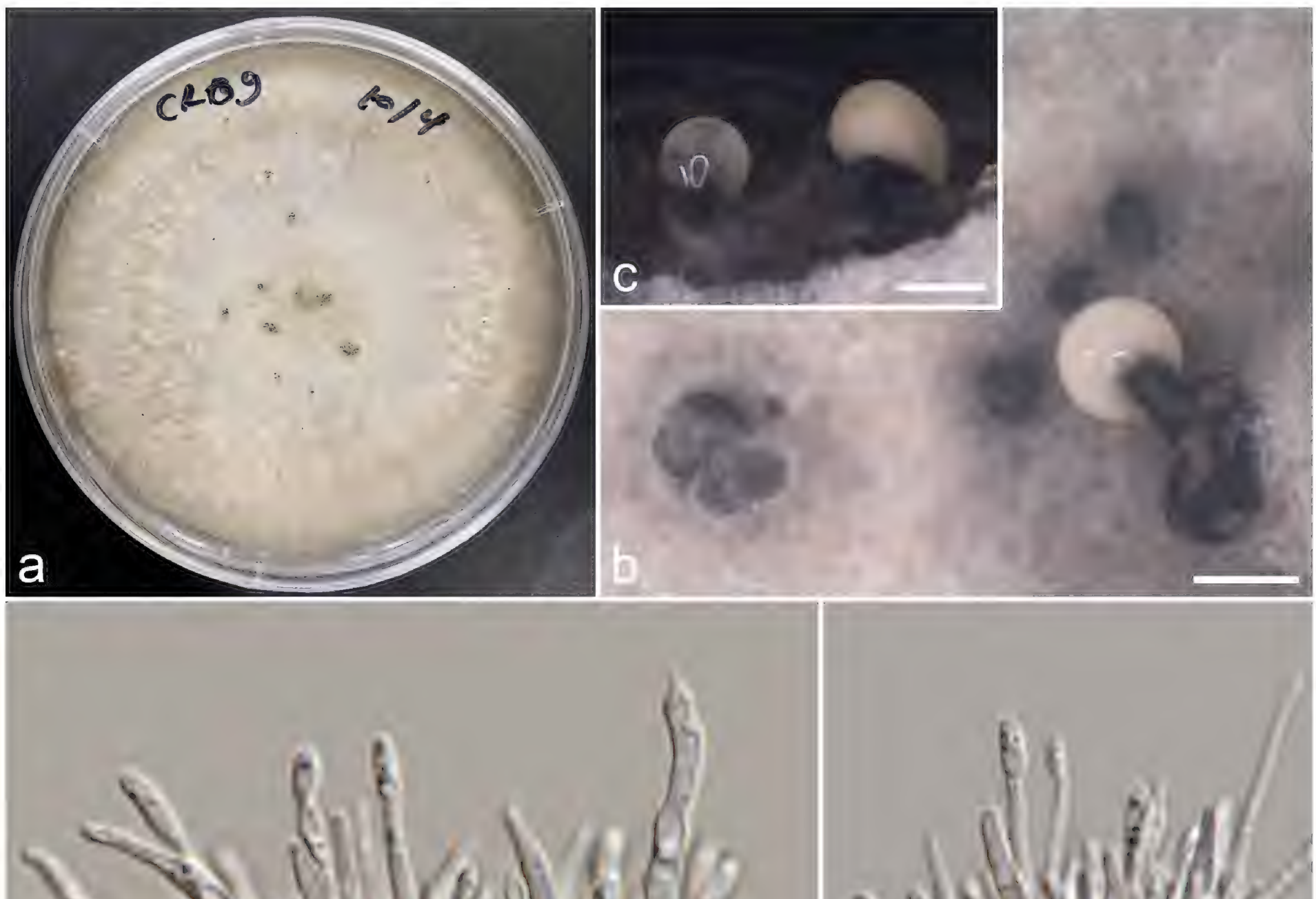


**Figure 4.** *Diaporthe thailandica* (Asexual morph, MFLUCC 24–0523) **a** culture on pda **b, c** conidiomata sporulating on pda **d, e** conidiogenous cells giving rise to conidia **f** alpha conidia **g** beta conidia **h** alpha and beta conidia. Scale bars: 500 µm **(b, c)**; 10 µm **(d–h)**.

**Material examined.** THAILAND, Chiang Rai Province, Muang District, on a dead unidentified dicot, 16 January 2023, J. Louangphan, CR1-09 (MFLU 23–0473, ***holotype***); ex-type MFLUCC 24–0523 = MFLUCC 23–0299.

**Host.** Unidentified branch (this study).

**Distribution.** Thailand (this study).

**Notes.** *Diaporthe thailandica* (MFLUCC 23-0299 and MFLUCC 24–0523) formed a sister clade with isolates of *D. raonikayaporum* (CBS 133182, MFLUCC 14–1133, and MFLUCC 14–1136) with 100% ML/1.00 BPP support (Fig. 1). *Diaporthe thailandica* differs from *D. raonikayaporum* in its conidiomata (500–700 × 300–600 µm *vs.* 110–200 × 50–130 µm), conidiophores (10–29.5 × 1.3–2.5 µm vs. 16–26 × 2–3 µm), conidiogenous cells (2.1–8.1 × 1–2.3 µm *vs.* 5–10 × 2–3 µm), and beta conidia (8.5–18.5 *vs.* 7–13 µm) (Gomes et al. 2013). *Diaporthe thailandica* differs from *D. raonikayaporum* (=*D. neoraonikayaporum* MFLUCC 14–1133) in its conidiomata (500–700 × 300–600 µm vs. 690–1190 × 805–1285 µm), conidiophores (10–29.5 µm *vs.* 15–23 µm), alpha conidia (5.3–8.8 × 2.3–3.5 µm *vs.* 4–6 × 2–3 µm), and beta conidia (8.5–18.5 µm *vs.* 13–21 µm) (Doilom et al. 2017). Gamma conidia was observed in *D. raonikayaporum* (= *D. neoraonikayaporum*) but not in *D. thailandica* (Doilom et al. 2017). *Diaporthe thailandica* further differs from *D. raonikayaporum*, which has only been reported as an asexual morph (Gomes et al. 2013; Doilom et al. 2017). Our strain differs significantly (> 2.5%) compared to the sequence data of *D. raonikayaporum* (Table 3). However, our isolate does not have *cal* sequence data, while *his3* sequence data is not available for *D. raonikayaporum* (MFLUCC 14–1133 and MFLUCC 14–1136). A pairwise homoplasy index showed Φw = 1.0 when a genealogical correlation model was applied between *Diaporthe thailandica* and *D. raonikayaporum* (Fig. 5). Thus, *Diaporthe thailandica* is reported as a new species based on morphology and molecular evidence.

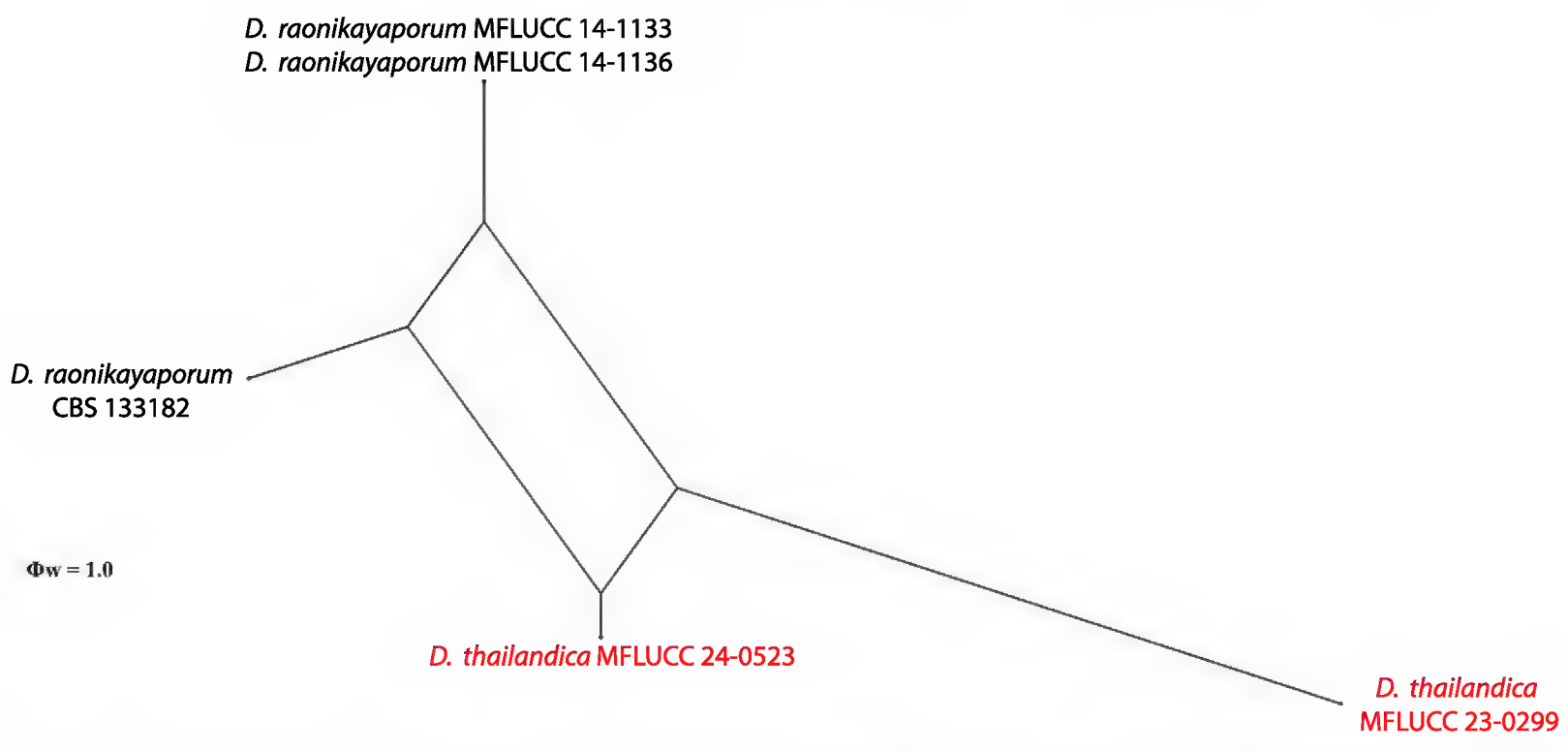


**Figure 5.** The splits graph from the pairwise homoplasy index (PHI) test generated from the combined ITS, *tef1*, *tub2*, *cal*, and *his3* sequence data of *Diaporthe thailandica* (indicated in red) and closely related taxa using both LogDet transformation and splits decomposition. PHI test results (Φw) < 0.05 indicate significant recombination within the dataset.

**Table 3.** Pairwise comparison of the sequences of *Diaporthe thailandica* and *D. raonikayaporum* isolates (excluding gaps).

| Sequences | *D. raonikayaporum* (CBS 133182) | *D. raonikayaporum* (MFLUCC 14-1133) | *D. raonikayaporum* (MFLUCC 14-1136) |
|---|---|---|---|
| ITS | 3.4% (18/529) | 2.6% (14/529) | 3.0% (16/529) |
| *tef1* | 6.5% (21/323) | 7.4% (24/323) | 8.4% (27/323) |
| *tub2* | 4.3% (18/415) | 6.5% (27/415) | 7.7% (32/415) |
| *cal* | – | – | – |
| *his3* | 3.7% (17/450) | – | – |

– Data not available.

***Diaporthe tulliensis*** **R.G. Shivas, Vawdrey & Y.P. Tan, Persoonia 35: 301 (2015)**
Index Fungorum: IF812896
Facesoffungi Number: FoF16300
Fig. 6

**Description.** ***Saprobic*** on decaying *Bambusa*. ***Sexual morph*:** Undetermined. ***Asexual morph*: *Conidiomata*** 91–148 × 311–974 µm ($\bar{x}$ = 120 × 583 µm, n = 20), pycnidial, scattered or aggregated, embedded in host surface, slightly erumpent through

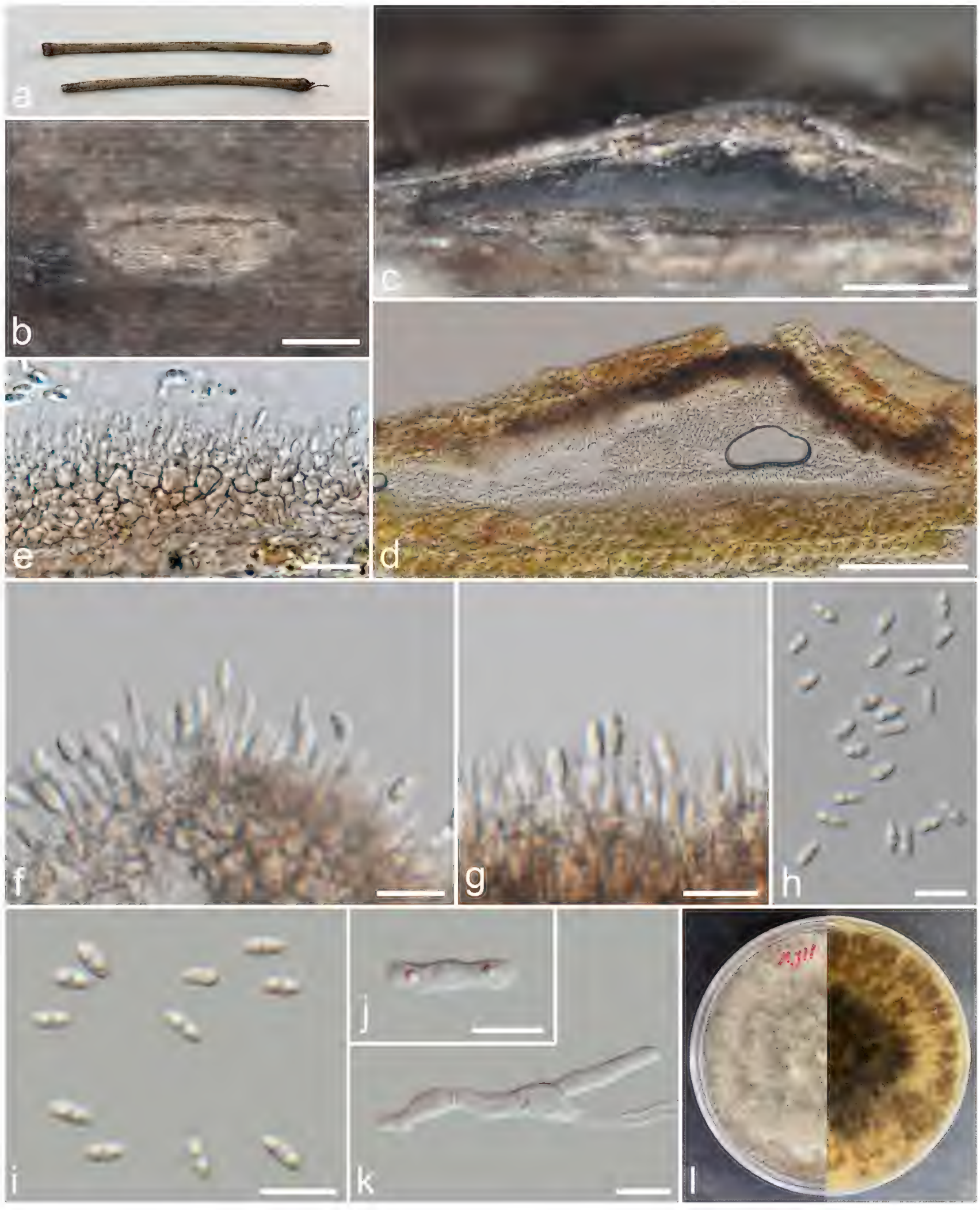


**Figure 6.** *Diaporthe tulliensis* (MFLU 23–0475) **a, b** conidiomata on host **c** longitudinal section through conidioma **d, e** transverse section of conidioma **f, g** conidiogenous cells giving rise to conidia **h, i** alpha conidia **j, k** germinated conidia **l** front and reverse view of the colony on PDA. Scale bars: 200 µm (**b–d**); 20 µm (**e**); 10 µm (**f–k**).

host surface, 1–3-locular conidioma, nearly flat, elongated, discoid, or variable in shape, black, consisting of hyaline, thin-walled cells of *textura angularis*, outer layer thick walled. ***Conidiophores*** reduced to conidiogenous cells. ***Conidiogenous cells*** 4–16 × 1.2–2.6 µm ($\bar{x}$ = 7.5 × 1.8 µm, n = 40), cylindrical, unbranched, aseptate, smooth, straight or slightly curved, tapering towards the apex, wider at base, hyaline. ***Alpha conidia*** 4.1–7.8 × 1.7–3.1 µm ($\bar{x}$ = 5.8 × 2.5 µm, n = 40), apex bluntly rounded, 1–2-guttulate, mostly bi-guttulate, oval or oblong to ellipsoid, hyaline, smooth, base obtuse to subtruncate. ***Beta*** and ***Gamma conidia*** not observed.

**Culture characteristics.** Colonies on PDA, reaching 40 mm diam., after 2 weeks at 25 °C, initially white, turning olivaceous grey after 7–10 days, darker at the centre and marginal area, lacking aerial mycelium; reverse, olivaceous grey bordered by dark margins.

**Material examined.** Thailand, Chiang Mai Province, Mae Taeng District, on dead terrestrial stem of *Bambusa* (Poaceae), 19 November 2022, J. Louangphan, MJ11 (MFLU 23–0475); living culture, MFLUCC 24–0524 = MFLUCC 23–0301.

**Hosts.** *Actinidia* spp. (Actinidiaceae), *Alangium kurzii* (Cornaceae), *Bambusa* sp. (Poaceae), *Bougainvillea glabra* (Nyctaginaceae), *Celtis formosana* (Ulmaceae), *Morinda officinalis* (Rutaceae), *Tectona grandis* (Lamiaceae), *Theobroma cacao* (Malvaceae), Soil, *Vitis vinifera* (Vitaceae) (Chang et al. 2005; Crous et al. 2015; Bai et al. 2017; Doilom et al. 2017; Yang et al. 2018; Manawasinghe et al. 2019; Tennakoon et al. 2021; Luo et al. 2022; this study).

**Distribution.** Australia, China, Korea, Thailand (Chang et al. 2005; Crous et al. 2015; Bai et al. 2017; Doilom et al. 2017; Yang et al. 2018; Manawasinghe et al. 2019; Tennakoon et al. 2021; Luo et al. 2022; this study).

**Notes.** In the phylogenetic analysis, our isolates (MFLUCC 23–0301 and MFLUCC 24–0524) clustered with *D. tulliensis* isolates (MFLUCC 14–1139, JZB320128, and BRIP 62248a) with 100% ML/1.00 BPP support (Fig. 1). Our isolate has a similar morphology to *D. tulliensis* isolates but differs from *D. tulliensis* in the size of conidiomata (up to 500 µm (= *D. celtidis* and *D. tulliensis*) *vs.* up to 510 µm (= *D. hubeiensis*) *vs.* 135–330 µm (= *D. alangii*) *vs.* 50–380 µm (= *D. morindae*) *vs.* 725–820 µm diam. (= *D. tectonae*)) (Crous et al. 2015; Doilom et al. 2017; Yang et al. 2018; Manawasinghe et al. 2019; Luo et al. 2022). Our isolate also differs due to the absence of beta conidia, which has been reported in some *D. tulliensis* isolates (Chang et al. 2005; Crous et al. 2015; Doilom et al. 2017; Manawasinghe et al. 2019). Therefore, we report our isolate as a new host record of *D. tulliensis*.

## Phylogenetic analyses of Xylariales

The phylogeny represents selected taxa in Xylariales based on the concatenated dataset of ITS, LSU, *rpb2*, and *tub2* sequences. The combined sequence alignment comprised 78 strains with 3624 characters, including gaps (ITS: 1–578, LSU: 579–1432, *rpb2*: 1433–2573, *tub2*: 2574–3624). The ML and BI analyses of single and multi-gene showed similar topologies. The best scoring ML tree with a final likelihood value of -54607.950 (Fig. 7). The matrix had 1992 constant sites, 1349 parsimony informative sites, and 2026 distinct site patterns. Estimated base frequencies were as follows: A = 0.2405, C = 0.2618, G = 0.2637, T = 0.2337, substitution rates: AC = 1.4137, AG = 3.9195, AT = 1.4543, CG = 1.1208, CT = 7.4615, GT = 1.000, gamma distribution shape parameter = 0.759631, and tree length = 7.506.

**Xylariales Nannf.**
**Xylariaceae Tul. & C. Tul.**
***Occultitheca* J.D. Rogers & Y.M. Ju**

***Occultitheca chiangraiensis* Louangphan, Phukhams., K.D. Hyde & Bhunjun, sp. nov.**
Index Fungorum: IF903235
Facesoffungi Number: FoF17287
Fig. 8

**Etymology.** The name refers to the province where the holotype was collected.

**Holotype.** MFLU 24–0414.

**Description.** ***Saprobic*** on early decaying branch. ***Sexual morph*: *Ascomata*** 220–342 × 228–395 µm ($\bar{x}$ = 290 × 324 µm, n = 15), immersed, solitary, scattered, globose to subglobose, erumpent through host surface, visible as black dot of ostiole, surrounded by a whitish halo. ***Clypeus*** carbonaceous, rudimentary, thick-walled, the ostiolar opening surrounded with black cells. ***Ostioles*** centric, ostiolar canal periphysate. ***Peridium*** 17–30 µm ($\bar{x}$ = 23 µm, n = 20) wide, tightly attached to the host tissue, with two cell layers, outer layer thick-walled, comprising yellowish brown cells of *textura angularis*, inner layer thin, composed of hyaline cells of *textura angularis*. ***Paraphyses*** 3.7–7.6 µm ($\bar{x}$ = 5.4 µm, n = 25) wide, wider at the base, longer than the asci, filamentous, septate, constricted at the septa, embedded in gelatinous matrix. ***Asci*** 112–158 × 8.5–13.7 µm ($\bar{x}$ = 131 × 11 µm, n = 25), 8-spored, unitunicate, cylindrical, short pedicellate, apically rounded, with 3.9–5.6 × 2.5–3.7 µm ($\bar{x}$ = 5 × 3 µm, n = 18), rectangular to slightly obconic, apical ring, J+ in Melzer's reagent. ***Ascospores*** 14.5–17.6 × 6–7.4 µm ($\bar{x}$ = 16.3 × 6.7 µm, n = 30), L/W 2.4, oblong to ellipsoidal, uniseriate, brown, inequilaterally unicellular, apical cell 13–15.5 µm ($\bar{x}$ = 14.5 µm, n = 30) long, usually with large guttules, brown cell with a mucilaginous sheath covering most of the spore length when mature, with a small, hyaline, rounded, basal cell, 1.3–2.3 µm ($\bar{x}$ = 1.8 µm, n = 30), lack of germ slit. ***Asexual morph*:** Undetermined.

**Culture characteristics.** Colonies on PDA, reaching 40 mm diam., after 21 days at 25 °C, circular, entire edge, smooth surface, flat, slightly woolly, smooth margin, above ash white from the centre to white at the edge with concentric rings of woolly; from below: light brown at the centre, white at the margin, with ash white mycelium.

**Material examined.** Thailand, Chiang Rai, Mae Fah Luang District, Mae Salong Nok, on a dead unidentified dicot branch, 16 January 2023, J. Louangphan, CR1–19 (MFLU 24–0414, holotype); ex-type MFLUCC 25–0158.

**Host.** Unidentified branch (this study).

**Distribution.** Thailand (this study).

**Notes.** Based on multi-gene phylogenetic analyses of ITS, LSU, *rpb2*, and *tub2* sequences, *Occultitheca chiangraiensis* (MFLU 24–0414) clustered with *O. rosae* (HKAS 102393) and *Clypeosphaeria oleae* (CPC 36779) with 100% ML and 1.00 BPP support (Fig. 7). *Clypeosphaeria oleae* was reported only from the asexual morph (Crous et al. 2019); thus, we could not compare the morphology between the species as we could not obtain the asexual morph of our strain, and therefore the link between them cannot be confirmed. Furthermore, *C. oleae* lacks *rpb2* and *tub2* data, which is important to confirm its

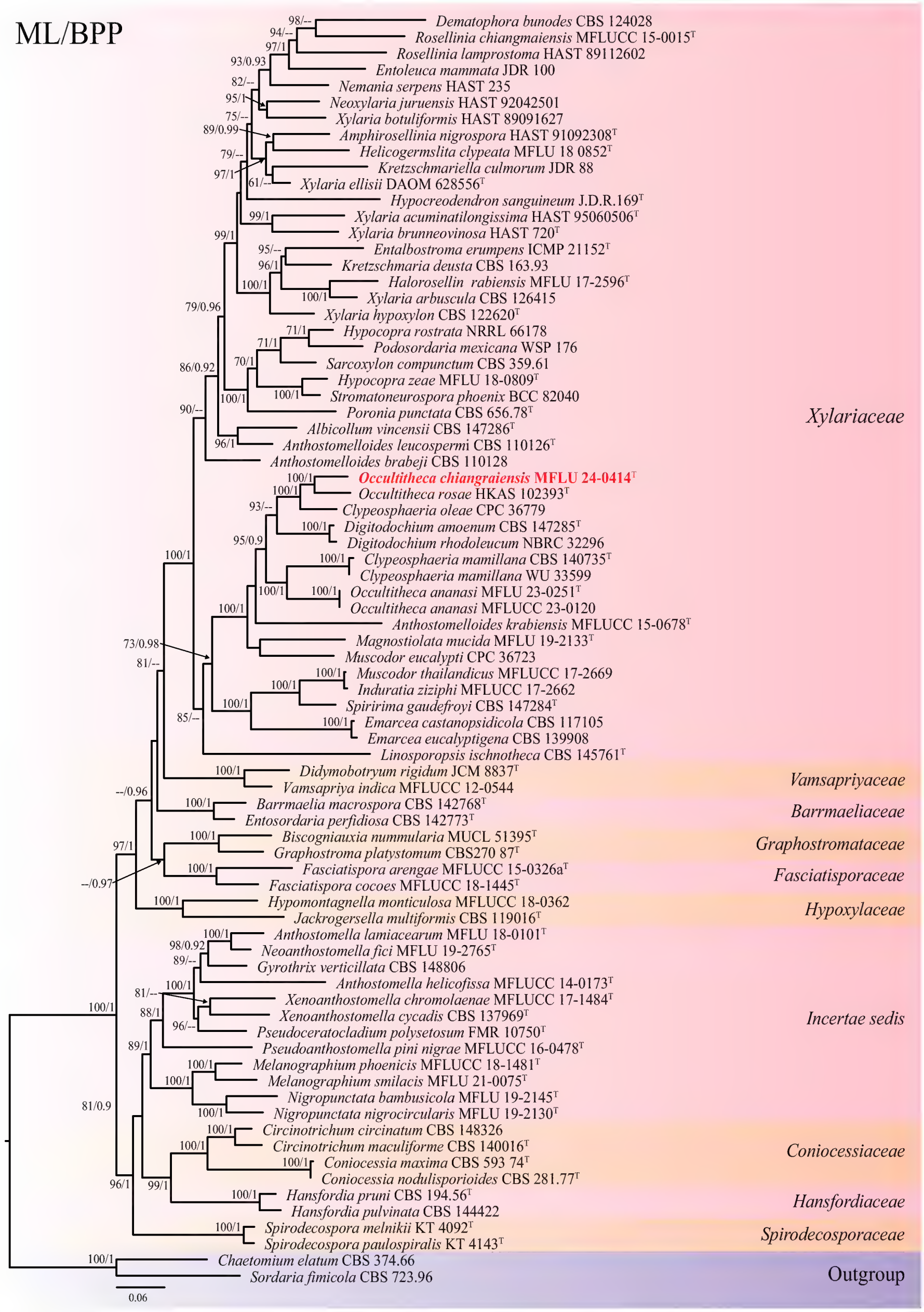


**Figure 7.** Phylogram generated from maximum likelihood analyses based on combined ITS-LSU-*rpb2-tub2* datasets. The tree is rooted with *Chaetomium elatum* (CBS 374.66) and *Sordaria fimicola* (CBS 723.96) as the outgroup taxa. Bootstrap support values ≥ 60% ML/≥0.90 BPP are given at the nodes. The newly generated taxa are indicated in red. The holotype/ex-type strains are denoted with T.

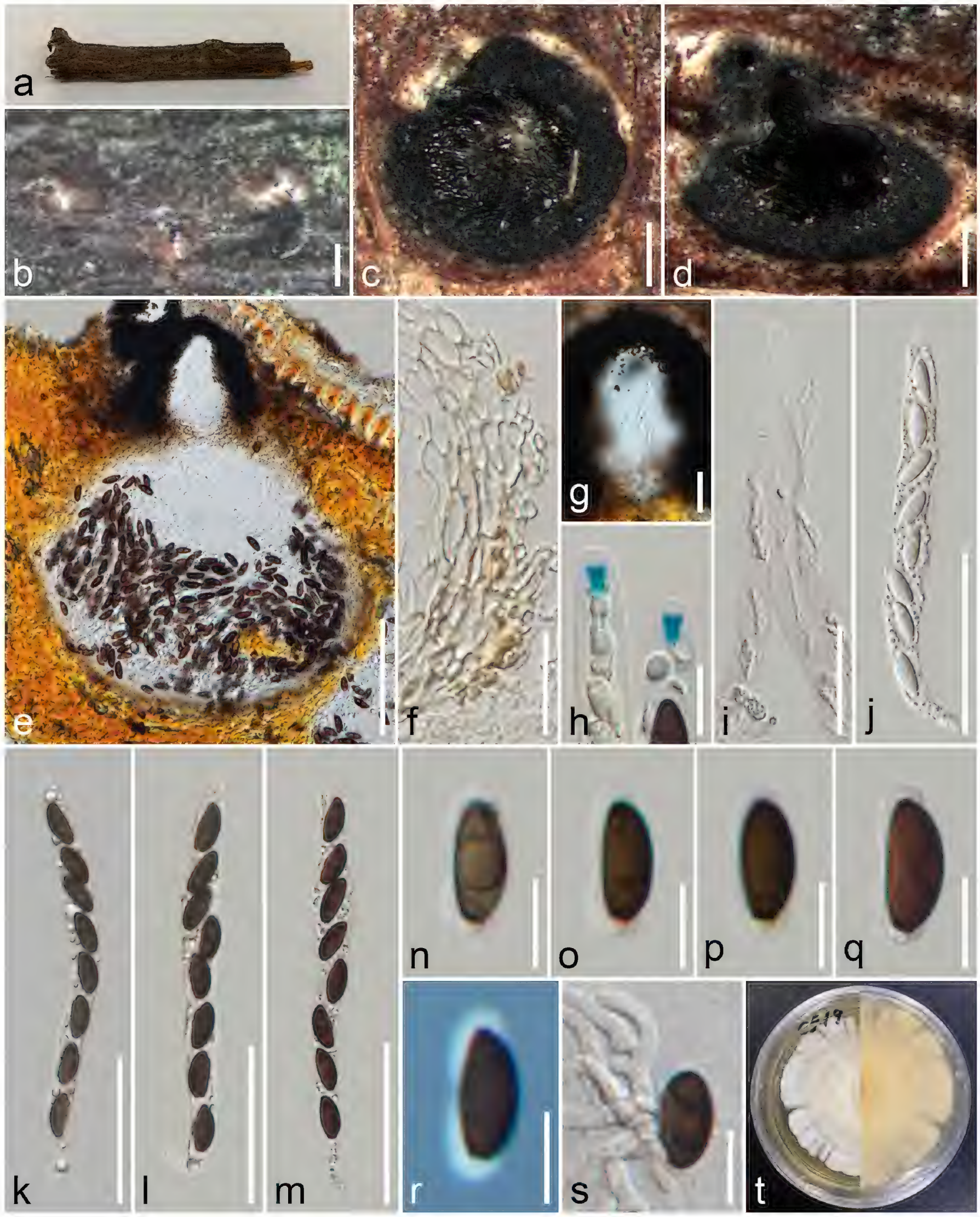


**Figure 8.** *Occultitheca chiangraiensis* (MFLU 24–0414, holotype) **a** host substrate **b** ascomata in host surface **c, d** horizontal and vertical section of ascoma **e** section through ascoma **f** peridium **g** ostiole **h** apical ring stained with melzer's reagent **i** paraphyses **j–m** immature and mature asci **n–r** ascospores (**r** ascospores show mucilaginous sheath in indian ink) **s** a germinated spore **t** front and reverse view of the colony on PDA. Scale bars: 200 µm (**b**); 100 µm (**c–e**); 20 µm (**f, g, i**); 10 µm (**h, n–s**); 50 µm (**j–m**).

phylogenetic placement. Morphologically, *Occultitheca chiangraiensis* fits the generic concept of *Occultitheca* in having immersed ascomata, short pedicellate asci with a J+, apical ring, a long distance between the ascus apex and the uppermost ascospore, and hyaline basal cells attached to brown ascospores

**Table 4.** Synopsis of *Occultitheca* species.

| Species | *O. costaricensis* | *O. rosae* | *O. ananasi* | *O. chiangraiensis* |
|---|---|---|---|---|
| Host | Unidentified decaying wood | *Rosa* sp. | *Ananas comosus* | Unidentified decaying wood |
| Country | Costa Rica | China | Thailand | Thailand |
| Ascomata (μm) | 400–600 | 360–385 × 350–420 | 190–230 × 160–260 | 220–342 × 228–395 |
| Peridium (μm) | – | 18–25 | 15–20 | 17–30 |
| Paraphyses (μm) | – | 3–6.5 | 3–5 | 3.7–7.6 |
| Asci (μm) | 185–190 × 10–10.5 | 90–140 × 11–13 | 70–90 × 5–10 | 112–158 × 8.5–13.7 |
| Apical ring (μm) | 6 × 3 | 3.5–4.5 × 2.8–3.2 | – | 3.9–5.6 × 2.5–3.7 |
| Ascospores (μm) | 14.5–23.5 × 7–10.5 | 16.5–20 × 6.5–8 L/W 2.6 | 10–12.5 × 3.5–4.5 L/W 2.9 | 14.5–17.6 × 6–7.4 L/W 2.4 |
| Basal cell (μm) | 1.5–4.5 | 1.5–2.2 | – | 1.3–2.3 |
| Spore sheath | No | Thin | Thin | One side thick |
| Germ slit | Straight | Straight | Straight | No |
| References | Rogers and Ju (2003) | Samarakoon et al. (2022) | Tian et al. (2024) | This study |

– Data not available.

(Rogers and Ju 2003; Samarakoon et al. 2022). Our isolate was compared to *Occultitheca* species as detailed in Table 4. *Occultitheca chiangraiensis* differs from *O. rosae* by having smaller ascomata ($\bar{x}$ = 290 × 324 μm vs. 370 × 385 μm), a lack of a germ slit, and possesses a thicker mucilaginous sheath compared to *O. rosae* (Samarakoon et al. 2022). Our strain differs from *O. ananasi*, which has uniseriate, olive-greenish ascospores becoming 2-seriate in the middle and thin mucilaginous sheath (Tian et al. 2024). *Occultitheca chiangraiensis* was also compared to the type species *O. costaricensis* as it lacks molecular data. *Occultitheca chiangraiensis* differs by having 1–2 individual ascomata and ascospores with a mucilaginous sheath, while *O. costaricensis* has 2–12 ascomata in a cluster and ascospores without a sheath (Rogers and Ju 2003). Additionally, some *Anthostomella* species have similar characteristics in terms of ascoma, asci, and ascospores with draft cells and lack germ slits, such as *A. clypeata* and *A. clypeoides*, but differ in a short space of the top ascospore and the ascus apex and shape of the sheath compared to *Occultitheca* species (Lu and Hyde 2000; Rogers and Ju 2003). Our strain differs by 6% in the ITS region (30/482, 4 gaps), 2% in LSU (14/745, 4 gaps), 15% in *rpb2* (117/798, no gap), 16% in *tub2* (120/754, 22 gap), and 3% in *tef1* (31/915, 1 gap) sequences compared to *O. rosae* (HKAS 102393). Thus, *Occultitheca chiangraiensis* is reported as a new species based on morphology and phylogenetic evidence.

## Discussion

In this study, two novel species, *Diaporthe thailandica* and *Occultitheca chiangraiensis*, along with a new host record of *D. tulliensis* and a new geographical record of *D. melonis*, are introduced based on morphology and molecular data. This study expands the known diversity of these taxa and highlights the importance of saprobic microfungi in ecological systems.

*Diaporthe* is a species-rich genus with a diverse host range and global distribution (Dissanayake et al. 2017; Phukhamsakda et al. 2020; Hongsanan et al. 2023; Bhunjun et al. 2024b). The species have overlapping morphological traits. There are more than 1200 epithets under *Diaporthe* in Index Fungorum (2024); thus, the boundaries of the species/species complexes within the genus have

been revised by several studies. *Diaporthe* was recently restructured into seven sections and 15 species complexes based on molecular analyses. Several *Diaporthe* species have been synonymised under *D. tulliensis* (*D. alangii*, *D. celtidis*, *D. glabrae*, *D. hubeiensis*, *D. morindae*, and *D. tectonae*), and these taxa formed a clade in our trees (including in the backbone tree with 226 taxa; data not shown), similar to Dissanayake et al. (2024). *Diaporthe melonis* (= *D. guangdongensis*) also formed a clade in our trees (including in the backbone tree; data not shown), similar to Dissanayake et al. (2024). The implementation of the markers (ITS, *tef-1*, and *tub*) proved to be phylogenetically informative in this study, resulting in a similar topology as previous studies based on five-marker combinations. The GCPSR analysis was also used to support the novelty of the *Diaporthe* species in this study. Therefore, molecular data and morphological evidence are needed for accurate species identification, thus reinforcing the importance of integrative approaches.

Morphologically, *Occultitheca* is considered an inconspicuous xylarialean and treated as anthostomella-like taxa in terms of having immersed, clypeate ascomata, asci with a J+, apical ring, ascospores with a large brown cell, and a basal hyaline dwarf cell (Daranagama et al. 2018; Samarakoon et al. 2022). Anthostomella-like taxa have now been split into five genera as suspected by Daranagama et al. (2015). These include *Anthocanalis*, *Astrocystis*, *Brunneiperidium*, *Lunatiannulus*, and *Pyriformiascoma*, and they differ in terms of ascomata and asexual morph characters. *Occultitheca* has a distinctive ostiole surrounded by a whitish halo and apiosporous ascospores with a small hyaline dwarf cell at one end and a dark brown, larger cell. There are only three species in *Occultitheca*, and all of them were found as saprobes as sexual morphs from terrestrial ecosystems. *Occultitheca* has a limited distribution and a narrow range of hosts, as only one species was found in Costa Rica (Rogers and Ju 2003), one in China, and one in Thailand (Samarakoon et al. 2022; Tian et al. 2024). Here, we provide a new addition to *Occultitheca* from unidentified decaying wood in Chiang Rai, Thailand. *Occultitheca chiangraiensis* was reported only as a sexual morph due to failure to obtain the asexual morphs from cultures. Thus, the link between the sexual and asexual morphs of this genus remains unknown. Due to the uncertainty of asexual and sexual morphologies and the low number of collections, *Occultitheca* has been placed under Xylariales genera *incertae sedis* (Samarakoon et al. 2022; Tian et al. 2024) and has also been considered to be part of Xylariaceae based on phylogenetic analyses (Voglmayr et al. 2022; this study). Expanding the sample collection will improve the representativeness of the genus.

This study provides a vital contribution to our understanding of Sordariomycetes diversity. Introducing new taxa is significant as they contribute to the broader understanding of fungal evolution, taxonomy, and ecology. It also contributes to the growing knowledge about the diversity of fungi associated with woody litter. It emphasises the necessity for continued exploration of fungal biodiversity across various habitats. As global ecosystems undergo rapid changes due to climate shifts and habitat destruction, understanding these dynamics will be crucial for conservation efforts and ecosystem management. Therefore, this study encourages further exploration in understudied substrates and regions, which could unveil additional species and enrich our comprehension of fungal ecology and taxonomy.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

The authors confirm contributions to the paper as follows: Fungal specimen collection and isolation, fungal specimen deposition, and manuscript writing: Chayanard Phukhamsakda, Johnny Louangphan, Kedsara Navasit, Chitrabhanu S. Bhunjun; fungal identification and contributed to the revision of the manuscript: Chayanard Phukhamsakda, Kevin D. Hyde, Milan C. Samarakoon, Fatimah O. Alotibi, Chitrabhanu S. Bhunjun. All authors have read and agreed to the published version of the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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